DATA SHEET

Quantitative
Bovine IgG ELISA
ZMC Catalog #: 0801198

INTENDED USE
The IMMUNO-TEK Bovine IgG ELISA Kit is a rapid, easy to use enzyme linked immunosorbent assay (ELISA) designed for the measurement of bovine IgG in bovine colostrum, milk, serum, plasma or other biological fluids. The assay contains mostly ready-to-use reagents and takes less than two hours to perform. The microplate and detector antibody in the kit have been specifically balanced to react uniformly with all subclasses of bovine IgG.

The IMMUNO-TEK Bovine IgG ELISA Kit is for Research Purposes Only.

PRINCIPLE OF THE TEST
Microplate wells coated with polyclonal antibodies to bovine IgG form the capture phase of the assay. These antibodies bind uniformly to all subclasses of bovine IgG. Captured bovine IgG then reacts with detector antibody, which is a polyclonal anti-bovine IgG, conjugated with horseradish peroxidase. This reagent also reacts uniformly with all subclasses of bovine IgG. Enzyme activity in the wells is then quantified using tetramethyl benzidine as a substrate.

REAGENTS

Materials Supplied:
Microplate, (1x96 well): Strips coated with purified goat anti-bovine IgG
Detector Antibody (12 ml): Contains conjugated goat anti-bovine IgG peroxidase
Bovine IgG Standard (7 ml): Contains bovine IgG and Assay Diluent
Assay Diluent (100 ml): Contains PBS, Triton X-100® and 2-chloroacetamide
Plate Wash Buffer (125 ml): Contains PBS, Tween 20® and 2-chloroacetamide
Substrate (12 ml): Contains Tetramethyl Benzidine (TMB)
Stop Solution (12 ml): Proprietary formulation
Microplate Sealers (1 pk): 10 sealers per pack
Plastic Bag (1 bag): For storage of unused microplate strips ® Triton X-100 is a registered trademark of Union Carbide Chemicals and Plastics Co., Inc. Tween 20 is a registered trademark of Imperial Chemical Industries.

Storage:
Store all kit reagents at 2-8°C. Do not freeze.

Materials required but not supplied:
Disposable gloves

Gentaur Molecular Products
Voortstraat 49
1910 Kampenhout, Belgium

Test tubes and racks for preparing specimen and IgG standard dilutions
Validated adjustable micropipettes, single and multi-channel
Distilled or deionized water
Validated incubator capable of maintaining 37 C+ 1 C
Graduated cylinders and assorted beakers
Validated microtiter plate reader
Automatic microtiter plate washer or manual vacuum aspiration equipment
Timer

PRECAUTIONS

FOR RESEARCH USE ONLY. Not For *in vitro* Diagnostic Use.
Prior to performing the assay, carefully read all instructions.
Use universal precautions when handling kit components and test specimens.*
To avoid cross-contamination, use separate pipette tips for each specimen.
When testing potentially infectious specimens, adhere to all applicable local, state and federal regulations regarding the disposal of biohazardous materials.
Stop Solution contains hydrochloric acid, which may cause severe burns. In case of contact with eyes or skin, rinse immediately with water and seek medical assistance. Wear protective clothing and eyewear.
*MMWR, June 24, 1988, Vol. 37, No. 24, pp. 377-382, 387-388

PREPARATION OF REAGENTS

Plate Wash Buffer
Dilute 10X Plate Wash Buffer 1:10 in distilled or deionized water prior to use. Mix thoroughly. Prepared 1X Plate Wash Buffer can be stored at 2-8 C for up to one week. Additional 10X Plate Wash Buffer (ZMC Catalog #: 0801060) may be ordered if needed.

Bovine IgG Standard Curve
Label 6 test tubes as shown in **Table**. The Bovine IgG Standard is provided at 125 ng/ml. This should be diluted in Assay Diluent as follows to prepare a standard curve.

Table Preparation of Bovine IgG Standards

Tube Number	Concentration of Bovine IgG	Volume of Bovine IgG Standard	Volume of Assay Diluent
1	125 ng/ml	1000 µl	0 µl
2	62.5 ng/ml	500 µl of #1	500 µl
3	31.25 ng/ml	500 µl of #2	500 µl
4	15.6 ng/ml	500 µl of #3	500 µl
5	7.8 ng/ml	500 µl of #4	500 µl
6	0 ng/ml	0 µl	500 µl

SPECIMEN DILUTIONS
Bovine colostrum will normally contain 50-80 mg/ml of IgG antibody. A 1:1,000,000 dilution of bovine colostrum in Assay Diluent is recommended for initial testing. Bovine milk will normally contain ~0.5 mg/ml of IgG antibody. A 1:10,000 dilution of bovine milk in Assay Diluent is recommended for initial testing. Bovine serum will normally contain 25-30 mg/ml of IgG antibody. A 1:500,000 dilution of bovine serum in Assay Diluent is recommended for

initial testing. After initial testing, it may be necessary to adjust the dilution of the specimen sample in order to obtain an IgG concentration between 125 ng/ml and 7.8 ng/ml for accurate quantification.

TEST PROCEDURE

Allow all reagents to reach room temperature before use. Label test tubes to be used for the preparation of standards and specimens. If the entire 96 well plate will not be used, remove surplus strips from the plate frame and place into the resealable Plastic Bag with desiccant. Seal bag and store at 2-8°C.

Step 1: Label each strip on its end tab to ensure identity should the strips become detached from the plate frame during the assay.
Step 2: Designate one well on the plate and leave empty. This well will serve as a substrate blank.
Step 3: Pipette **200 l** of **standards 1-6** into duplicate wells.
Step 4: Pipette **200 l** of each **specimen** into duplicate wells.
Step 5: Cover the microplate with a plate sealer and incubate the plate for 30 minutes at 37 C.
Step 6: Aspirate the contents of each well and wash the wells 4 times with 1X Plate Wash Buffer. To wash, fill the wells with **300 l of 1X plate wash buffer** and aspirate. Perform 4 fill/aspirate cycles. After the final wash cycle, thoroughly blot the plate by carefully striking the plate on a pad of absorbent paper towels. Continue until no visible droplets of Plate Wash Buffer are observed.
Step 7: Pipette **100 l** of **Detector Antibody** into each standard and specimen well. **Do not add Detector Antibody to the substrate blank well.**
Step 8: Cover the plate with a plate sealer and incubate for 30 minutes at 37 C.
Step 9: Wash the plate 4 times with Plate Wash Buffer as described in **Step 6.**
Step 10: Pipette **100 l** of **Substrate** into each well **including** the substrate blank well.
Step 11: Incubate the plate for 30 minutes at room temperature. A blue color will develop in wells containing bovine IgG.
Step 12: Pipette **100 l** of **Stop Solution** into each well. A color change from blue to yellow will occur.
Step 13: Within 15 minutes, read the optical density of each well at **450 nm** using a microtiter plate reader.

Test Validity:
For the test to be valid, the mean optical density of the 0 ng/ml standard and the substrate blank must be below 0.200.

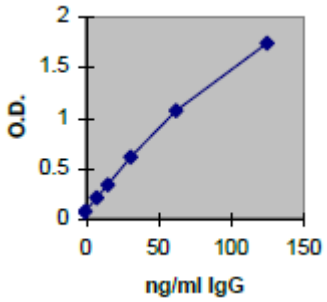
CALCULATION AND INTERPRETATION OF RESULTS

Using linear graph paper or a computer program, plot the optical densities of each standard on the Y-axis versus the corresponding concentration of the standards on the X-axis. The concentration of bovine IgG in each diluted specimen may then be determined manually using a ruler to extrapolate, by linear regression using a computer program or pocket calculator with a linear regression function, or by point-to-point calculation again using a computer or calculator. Correct the diluted specimen values by the dilution factor used to obtain the final concentration of bovine IgG in the original specimen.

Typical Standard Curve

Below is an example of a typical standard curve. Variations will occur from laboratory to laboratory due to pipetting, incubator temperatures, plate readers, etc.

Bovine IgG Standard Concentration	Optical Density at 450 nm
125 ng/ml	1.736
62.5 ng/ml	1.064
31.25 ng/ml	0.603
15.6 ng/ml	0.329
7.8 ng/ml	0.198
0 ng/ml	0.063
Substrate Blank	0.048



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